Journal of the American College of Cardiology Vol. 47, No. 11, 2006

Published by Elsevier Inc.
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2006.01.066

Randomized Controlled Trial of the Effects of Remote Ischemic Preconditioning on Children Undergoing Cardiac Surgery

First Clinical Application in Humans

Michael M. H. Cheung, MB, ChB, MRCP, Rajesh K. Kharbanda, MD, PhD, Igor E. Konstantinov, MD, PhD, Mikiko Shimizu, MD, Helena Frndova, Meng, Jia Li, MD, PhD Helen M. Holtby, MD, Peter N. Cox, MD, Jeffrey F. Smallhorn, MD, FRCP, Glen S. Van Arsdell, MD, Andrew N. Redington, MD, FRCP

Toronto, Canada

OBJECTIVES We conducted a randomized controlled trial of the effects of remote ischemic preconditioning (RIPC) in children undergoing repair of congenital heart defects.

BACKGROUND Remote ischemic preconditioning reduces injury caused by ischemia-reperfusion in distant organs. Cardiopulmonary bypass (CPB) is associated with multi-system injury. We hypothesized that RIPC would modulate injury induced by CPB.

METHODS Children undergoing repair of congenital heart defects were randomized to RIPC or control treatment. Remote ischemic preconditioning was induced by four 5-min cycles of lower limb ischemia and reperfusion using a blood pressure cuff. Measurements of lung mechanics, cytokines, and troponin I were made pre- and postoperatively.

RESULTS Thirty-seven patients were studied. There were 20 control patients and 17 patients in the RIPC group. The mean age and weight of the RIPC and control patients were not different (0.9 ± 0.9 years vs. 2.2 ± 3.4 years, $p = 0.4$; and 6.9 ± 2.9 kg vs. 11.5 ± 10 kg, $p = 0.06$). Bypass and cross-clamp times were not different (80 ± 24 min vs. 88 ± 25 min, $p = 0.3$; and 55 ± 13 min vs. 59 ± 13 min, $p = 0.4$). Levels of troponin I postoperatively were greater in the control patients compared with the RIPC group ($p = 0.04$), indicating greater myocardial injury in control patients. Postoperative inotropic requirement was greater in the control patients compared with RIPC patients at both 3 and 6 h (7.9 ± 4.7 vs. 10.9 ± 3.2, $p = 0.04$; and 7.3 ± 4.9 vs. 10.8 ± 3.9, $p = 0.03$, respectively). The RIPC group had significantly lower airway resistance at 6 h postoperatively ($p = 0.009$).

CONCLUSIONS This study demonstrates the myocardial protective effects of RIPC using a simple noninvasive technique of four 5-min cycles of lower limb ischemia and reperfusion. These novel data support the need for a larger study of RIPC in patients undergoing cardiac surgery. (J Am Coll Cardiol 2006;47:2277–82) © 2006 by the American College of Cardiology Foundation

Ischemic preconditioning is an innate protective mechanism that markedly reduces ischemia-reperfusion (IR) injury in most human tissues. Although local ischemic preconditioning, induced by short-lived non-fatal ischemia in the target tissue, has been shown to be of benefit in patients undergoing coronary angioplasty and surgical revascularization in some studies (1,2), other studies (3,4) have been less conclusive. Furthermore, the clinical applicability of local preconditioning is limited by the need to induce ischemia in the target organ, a process that itself may induce dysfunction and that is clearly inappropriate for global myocardial protection.

A more clinically relevant stimulus is afforded by remote ischemic preconditioning (RIPC). The concept of RIPC was first described by Przyklenk et al. (5). Transient ischemia of the left circumflex territory was shown to reduce the effects of subsequent potentially lethal ischemia in the left anterior descending artery territory in dogs. Further studies in rodent models demonstrated that ischemia of the kidney and intestine may induce myocardial protection (6,7). Although providing proof of principle, none of these studies has particular relevance to protection against IR injury in the clinical setting. We have recently demonstrated (8) that skeletal muscle ischemia is a potent preconditioning stimulus in humans and larger animals. Four 5-min episodes of limb ischemia induced by inflation of a blood-pressure cuff prevented ischemic endothelial dysfunction in the forearm in normal volunteers and reduced infarct size in a porcine model of myocardial infarction. The same stimulus, when applied to the recipient, protects the donor heart against IR injury in a cardiac transplant model (9) and has been shown to modify expression of proinflammatory genes in circulating human neutrophils (10). Furthermore, in a porcine model of cardiopulmonary bypass (CPB), RIPC afforded myocardial and pulmonary protection (11,12). This was evidenced by lower levels of troponin I and shorter duration of lactic acidosis, in addition to lower pulmonary vascular resistance, and significantly less change in pulmo-

From the Divisions of Cardiology and Cardiovascular Surgery, Critical Care Medicine and Anaesthesia, Hospital for Sick Children, Toronto, Canada. This work was supported by the Heart and Stroke Foundation of Canada and the Canadian Institute of Health Research.

Manuscript received November 8, 2005; revised manuscript received January 10, 2006, accepted January 16, 2006.

JACC Vol. 47, No. 11, 2006
June 6, 2006:2277–82

Abbreviations and Acronyms

A-a O_2 = alveolar-arterial oxygen
CPB = cardiopulmonary bypass
ECG = electrocardiographic/electrocardiogram
IL = interleukin
IR = ischemia-reperfusion
RIPC = remote ischemic preconditioning
TNF = tumor necrosis factor

nary vascular resistance and lower peak inspiratory pressure after CPB in comparison with control patients. Open-heart surgery in children results in a predictable IR injury with a well-documented systemic inflammatory reaction. We therefore hypothesized that RIPC would provide protection against myocardial IR injury and systemic inflammation in children undergoing CPB for repair of congenital heart defects.

METHODS

The study protocol was approved by the institutional research ethics board. Informed consent was obtained before enrollment in the study. Children undergoing surgical repair of congenital heart defects were randomized to RIPC or a control group. Staff involved in the clinical care and members of the study group obtaining functional data were blinded to randomization for the period of data acquisition and analysis. Group allocation was not revealed until the final statistical analysis. All subjects were studied under general anesthesia at the time of surgical repair. Children undergoing all types of open-heart surgery were included, except for those with isolated atrial septal defect and those with a bidirectional cavopulmonary shunt undergoing Fontan completion. These patient groups were excluded because bypass times are relatively short and duration of stay in the intensive care unit is often <24 h. Patients with chromosomal defects, airway and parenchymal lung disease, immunodeficiency, or blood disorders were excluded. Anesthesia was induced with sevoflurane and maintained with a combination of intravenous fentanyl and inhaled isoflurane in air and oxygen. Muscle relaxants were used in all patients. Following insertion of central venous and arterial catheters and a 5-min period of stabilization, the following baseline measurements were made before sternotomy.

Lung function. Airflow and airway opening pressure were measured with a fixed-orifice differential flow sensor (CO2SMO Plus, Novametrix Medical Systems Inc., Wallingford, Connecticut) inserted between the endotracheal tube and the ventilator Y-piece. Recordings of dynamic compliance and airway resistance were stored in the memory of the CO2SMO Plus.

Blood analysis. Arterial blood was sampled from the arterial catheter for measurements of the cytokines interleukin (IL)-6, IL-8, and IL-10, tumor necrosis factor (TNF)-alpha, and levels of troponin I. Samples were collected and immediately centrifuged, and the resulting plasma and serum was frozen at −70°C for later analysis. Analysis of levels of cytokines and troponin I was made using commercially available kits (Immulite, Diagnostic Products Corporation, California, and Bayer Immuno 1, Bayer AG, Germany) respectively.

Total body water. A gross assessment of the systemic inflammatory response and the associated capillary leak was made by measuring changes in total body water. Two pairs of electrocardiographic (ECG) electrodes were applied to the arm and leg. Body resistivity was measured using a Bodystat 1500 (Bodystat, Isle of Man, United Kingdom). The principle underlying the non-invasive technique of bio-impedance analysis is the difference in resistivity of fluid within the body compared with lean tissue. The method has been validated and has been used following cardiopulmonary bypass in children (13).

Remote preconditioning protocol. Remote ischemic preconditioning was induced by four 5-min cycles of lower limb ischemia and 5-min reperfusion using a blood-pressure cuff inflated to a pressure 15 mm Hg greater than the systolic arterial pressure measured via the arterial line. Control patients underwent sham placement of the blood pressure cuff around the leg without inflation. There was a 5- to 10-min interval between completion of the RIPC protocol and initiation of bypass.

Surgical repair. All children underwent surgical repair using standard cardiopulmonary bypass techniques with blood cardioplegia. The duration of cardiopulmonary bypass and aortic cross-clamp time was recorded. None of the patients studied received perioperative steroids. Modified ultrafiltration was carried out in all children.

Postoperative assessment. All measurements were repeated at 3, 6, 12, and 24 h after bypass, with additional measurements of mixed venous saturation and urine output. Alveolar-arterial oxygen (A-a O_2 gradient) and oxygenation index were calculated in the usual way. Inotropic support at each time point was quantified by calculating the inotropic score as described previously (14,15).

Statistical analysis. Data are presented as mean ± SD, except in figures where error bars represent SEM. Data were compared by two-way ANOVA (Model 1, fixed effects) or unpaired *t* test, with the analysis of variance assessed by an F test. All tests were two-tailed. A p value <0.05 was considered significant. Data were analyzed using GraphPad Prism (version 4.0, GraphPad Software Inc., San Diego, California).

RESULTS

Patients. Thirty-seven patients were studied. No local adverse effects related to the RIPC stimulus were observed. There were 20 control patients and 17 in the RIPC group. Patients with ventricular septal (8 control and 7 RIPC) and atrioventricular septal defects (1 control and 3 RIPC), tetralogy of Fallot (10 control and 5 RIPC), aortic regurgitation undergoing valve repair (1 RIPC), and transposition

of the great arteries (1 in each group) were studied. The mean age and weight of the RIPC and control groups were not significantly different (0.9 ± 0.9 years vs. 2.2 ± 3.4 years, p = 0.4; and 6.9 ± 2.9 kg vs. 11.5 ± 10.1 kg; p = 0.06). Mean bypass and cross-clamp times were not significantly different (80 ± 24 min vs. 88 ± 25 min, p = 0.3 and 55 ± 13 min vs. 59 ± 13 min; p = 0.4). No patients were left with hemodynamically significant residual lesions. Length of stay in intensive care for RIPC and control groups was not significantly different (54.2 ± 40.7 h vs. 39.5 ± 25.7 h, respectively; p = 0.3). Duration of ventilation for the RIPC and control groups was not significantly different (30.4 ± 33.8 h vs. 25.0 ± 36.6 h, respectively; p = 0.6).

Myocardial function and injury. Levels of troponin I postoperatively were significantly greater in the control patients as compared with the RIPC group (p = 0.04), indicating greater myocardial injury in controls (Fig. 1). Because previous studies have shown greater release of troponin I in patients undergoing resection of muscle bundles associated with right ventricular outflow tract obstruction in tetralogy of Fallot (16), these data were reanalyzed excluding the data from this group of patients, and the difference between controls and RIPC patients remained significantly different (p = 0.04).

There was no difference in values of mixed venous saturation or urine output between the two groups throughout the postoperative period (Table 1). There was, however, a significantly greater inotropic requirement in the control group compared with the RIPC group at both 3 and 6 h (7.9 ± 4.7 μg/kg/min vs. 10.9 ± 3.2 μg/kg/min, p = 0.04; and 7.3 ± 4.9 μg/kg/min vs. 10.8 ± 3.9 μg/kg/min, p = 0.03, respectively) (Fig. 2).

Lung function. Airway resistance and dynamic compliance at baseline and postoperative time intervals are shown in Table 1. There were no significant differences in the baseline or 3-h postoperative values for airway resistance; however, the RIPC group had significantly lower airway resistance at 6 h postoperatively (p = 0.009) (Fig. 3). There

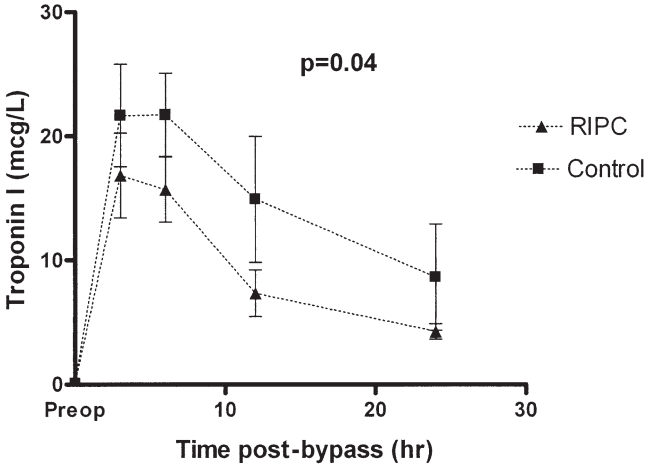

Figure 1. Pre- and postoperative levels of troponin I in remote ischemic preconditioning (RIPC) and control groups.

Table 1. Outcome Data

	Preop Control	Preop RIPC	3 h Postop Control	3 h Postop RIPC	6 h Postop Control	6 h Postop RIPC	12 h Postop Control	12 h Postop RIPC	24 h Postop Control	24 h Postop RIPC
Urine output (ml/kg/h)			2.6 ± 1.4	3.1 ± 2.1	2.0 ± 0.9	2.4 ± 1.2	1.5 ± 0.4	1.7 ± 0.6	1.5 ± 0.4	1.5 ± 0.4
Mixed venous saturation (%)			66 ± 8	64 ± 12	63 ± 10	64 ± 9	64 ± 8	64 ± 10	63 ± 8	63 ± 8
Lactate (mmol/l)			1.7 ± 0.5	1.9 ± 0.7	1.7 ± 0.7	1.5 ± 0.5	1.1 ± 0.3	1.5 ± 0.9	1.1 ± 0.3	1.4 ± 0.6
Bioimpedence (Ω)	803 ± 162	756 ± 93	755 ± 92	772 ± 114	690 ± 118	712 ± 117	669 ± 85	662 ± 132	640 ± 182	682 ± 113
Dynamic compliance (ml/cmH_2O)	5.3 ± 2.5	12.5 ± 11.9	4.9 ± 2.3	9.9 ± 8.4	5.0 ± 2.4	14.0 ± 20	4.8 ± 1.7	11.3 ± 12.6	5.5 ± 2.8	4.0 ± 1.2
Airway resistance (cm H_2O/ml/s)	51 ± 16	41 ± 19	47 ± 12	42 ± 20	49 ± 14†	34 ± 15†	53 ± 19	42 ± 17	47 ± 21	55 ± 13
Inotrope score (μg/kg/min)			11.4 ± 3.8*	8.1 ± 4.4*	11.0 ± 3.9*	7.8 ± 4.5*	10.1 ± 4.8	10.7 ± 6.9	9.2 ± 3.8	11.1 ± 6.5
A-a gradient (mm Hg)			129 ± 137	101 ± 102	77 ± 43	73 ± 88	62 ± 41	99 ± 114	110 ± 80	109 ± 137
Oxygenation index			3.3 ± 3.6	2.9 ± 2.8	2.3 ± 0.8	2.9 ± 2.8	2.5 ± 0.8	3.7 ± 3.2	3.3 ± 2.1	4.3 ± 4.6
IL-6 (pg/ml)	1.9 ± 1.3	1.6 ± 0.8	909 ± 1,860	1077 ± 2,100	369 ± 463	379 ± 462	325 ± 317	423 ± 604	126 ± 104	134 ± 111
IL-8 (pg/ml)	7 ± 4	6 ± 3	650 ± 1,567	634 ± 1,554	233 ± 481	195 ± 363	118 ± 221	47 ± 52	43 ± 52	43 ± 60
IL-10 (pg/ml)	6 ± 2	6 ± 1	46 ± 44	53 ± 69	33 ± 35	28 ± 26	36 ± 46	13 ± 9	12 ± 12	15.8 ± 27
TNF-α (pg/ml)	9 ± 3	8 ± 3	37 ± 37	33 ± 42	63 ± 170	20 ± 20	13 ± 8	10 ± 7	11 ± 7	9 ± 5

*p < 0.05 for control group vs. RIPC group. †p < 0.01 for control group vs. RIPC group.
A-a = alveolar-arterial; IL = interleukin; RIPC = remote ischemic preconditioning; TNF = tumor necrosis factor.

JACC Vol. 47, No. 11, 2006
June 6, 2006:2277–82

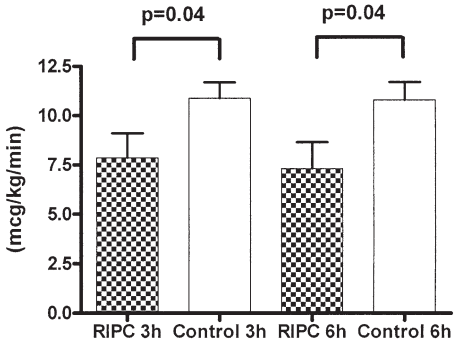

Figure 2. Postoperative inotrope scores in remote ischemic preconditioning (RIPC) and control groups.

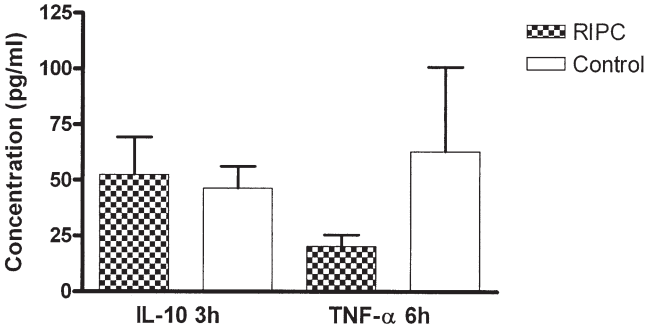

Figure 4. Levels of interleukin (IL)-10 at 3 h and tumor necrosis factor (TNF)-alpha at 6 h postoperatively in remote ischemic preconditioning (RIPC) and control groups.

was no significant difference in postoperative values of dynamic compliance between the two groups. A-a O_2 gradient and oxygenation index were not significantly different between the two groups (Table 1).

Systemic inflammatory response. Mean levels of the cytokines IL-6, IL-8, IL-10, and TNF-alpha at 3 and 6 h were not significantly different for the two groups of patients (Table 1). There was increased variance of IL-10 and a reduction in the variance of levels of TNF-alpha in the RIPC group. These were significantly different for IL-10 at 3 h ($p = 0.03$) and TNF-alpha at 6 h ($p < 0.0001$) (Fig. 4).

There were no differences in values of bioimpedence for the two groups (Table 1).

DISCUSSION

This study is the first demonstration of the clinical effectiveness of RIPC. Our simple protocol of transient limb ischemia provided protection against myocardial and pulmonary IR injury and also modulated the systemic inflammatory response in children undergoing open-heart surgery.

Clinical studies of local preconditioning. Clinical studies of local ischemic preconditioning in patients undergoing coronary angioplasty have shown conflicting data. A study by Bolli et al. (3) reported the beneficial protective effect of preconditioning in humans. In their study of patients undergoing coronary angioplasty, balloon inflations after the initial "preconditioning" inflation were associated with less diastolic and systolic dysfunction, less ST-segment elevation on intracoronary ECG, and less myocardial lactate production. Furthermore, in those patients given adenosine before angioplasty, there was amelioration of the effects of the first balloon inflation in comparison with control patients. The authors presented these data to support the existence of preconditioning in humans and that preconditioning could be induced by both direct myocardial ischemia and adenosine. In contrast, a randomized double-blind trial of the effects of the adenosine triphosphate-sensitive potassium channel opener pinacidil, the potassium channel blocker glibenclamide, and placebo during coronary angioplasty showed no evidence of ischemic preconditioning in humans (4). Using a similar angioplasty protocol to that of Bolli et al. (3), we found no significant differences in ECG ST-segment changes, ventricular function assessed by radionuclide detector, or symptoms of angina. The situation in humans is clearly more complicated than the more controlled environment of animal studies. The effects of comorbidity such as diabetes, hypertension and subsequent therapy, and the potential for a "preconditioning" effect of occult myocardial ischemia before angioplasty all conspire to make interpretation of the results of these studies difficult.

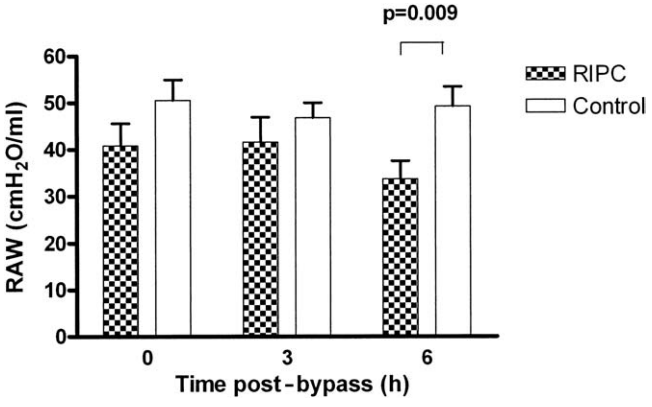

Figure 3. Airway resistance (RAW) in remote ischemic preconditioning (RIPC) and control groups.

Reduction in degree of myocardial necrosis due to prior local ischemic preconditioning has been demonstrated in patients undergoing coronary artery bypass grafting. Yellon et al. (2) reported significant reduction in troponin T release in those patients randomized to receive local ischemic preconditioning by two 3-min periods of aortic cross-clamping and rapid pacing, each followed by 2 min of reperfusion. As the authors pointed out, however, there is reluctance to subject patients with an already tenuous circulation to this type of stress, which has been well described to induce ventricular dysfunction. This is also relevant to the applicability of local ischemic preconditioning of the heart in children before repair of congenital defects.

One of the obvious advantages of our technique of remote preconditioning is its non-invasive nature and ease of application. Furthermore, in contrast to local ischemic preconditioning, the effects of transient skeletal muscle

ischemia are relatively benign, there being no myocardial dysfunction, risk of arrhythmia, low cardiac output, or secondary organ injury. Additionally, the "non-local" effect of RIPC may afford more widespread protection against IR injury and the CPB-induced systemic inflammatory response.

Remote preconditioning and the systemic inflammatory response. Ischemia-reperfusion injury and CPB during cardiac surgery are associated with a predictable systemic inflammatory response, myocardial dysfunction, and pulmonary endothelial dysfunction. These events may contribute to postoperative morbidity and mortality in any patient after heart surgery, but are particularly prominent in infants undergoing repair of congenital lesions. First, CPB itself, because of contact of blood with the extracorporeal circuit and direct trauma to its cellular constituents, drives a neutrophil-mediated inflammatory response. Second, IR injury of the cardiopulmonary bed during aortic cross-clamping leads to a secondary inflammatory response and concomitant alterations in gene expression affecting both the inflammatory process and pathways controlling programmed cell death or apoptosis. Our previous study of human neutrophils following RIPC, induced by four 5-min cycles of upper-limb ischemia, demonstrated modulation of genes coding for key proteins involved in cytokine synthesis, leukocyte chemotaxis, adhesion and migration, exocytosis, innate immunity signaling pathways, and apoptosis (10). This led to the hypothesis that organ injury associated with cardiopulmonary bypass might be reduced.

In this study, we did not observe any significant difference in the mean levels of IL-6, IL-8, IL-10, or TNF-alpha. There were, however, even within this relatively small group of patients, significant differences in variance of levels of IL-10 and TNF-alpha. The deleterious effects of high levels of TNF-alpha; induced by CPB are well described, including myocardial depression (17), capillary leak, and pulmonary dysfunction (18). Furthermore, in vivo dog experiments have demonstrated time-dependent myocardial dysfunction following intravenous infusion of TNF-alpha (19,20). Importantly, the overall response is modulated by the anti-inflammatory cytokine IL-10 (21) through inhibition of expression of TNF-alpha. The current data are consistent with an acute modification of inflammatory pathways with RIPC. It should be pointed out, however, that in our previous study, whereas the anti-inflammatory gene responses were significantly down-regulated at 15 min after the RIPC stimulus, the responses were markedly amplified at 24 h. These data are consistent with the well-described phenomenon of a second window (starting approximately 24 to 48 h after the stimulus) of protection afforded by ischemic preconditioning. Although this is speculative, one might therefore anticipate that further beneficial modification of the inflammatory response to cardiopulmonary bypass may occur if the RIPC stimulus were applied one day before surgery. Further studies are clearly required to explore this, however.

Lung function. Endothelial dysfunction and impairment of mechanical properties of the lung following CPB are well described (22–25). These effects are mediated by neutrophil activation leading to the release of proteases and oxygen radical species. Although neutrophil function was not assessed in this study, it has been shown previously (26) that RIPC reduced neutrophil activation and endothelial dysfunction in a human forearm model of IR-induced injury. Furthermore, in a human study of aortic cross-clamping to precondition the heart before valve replacement, it was noted that the increase in leukocyte numbers, thromboxane B2, and malondialdehyde levels in pulmonary venous blood from the lung was attenuated by preconditioning of the heart (27). There was also less lung injury histologically and a lower lung leukocyte count compared with control patients without myocardial preconditioning. These data may represent inadvertent remote preconditioning of the lungs and are consistent with our observations of differences in airway resistance postoperatively in this study and our findings of lower pulmonary vascular resistance and improved lung mechanics in a porcine model of CPB (11).

Myocardial injury and ventricular function. Our previous study (8) showed that RIPC induced by a similar protocol of limb ischemia reduced myocardial IR injury in a porcine model of myocardial infarction induced by balloon coronary occlusion. Congenital heart surgery is associated with a predictable global myocardial IR insult as a result of aortic cross-clamping. We have previously shown (28) a measurable degree of left ventricular dysfunction even after repair of "simple" congenital defects. Furthermore, many studies have demonstrated global myocardial damage using surrogate markers such as troponin I and T. In the current study, we used levels of troponin I as our primary measure of myocardial injury. Control patients had significantly higher levels of troponin I release in the postoperative period. The variation in troponin I levels at each time point for RIPC patients was also reduced in comparison with control patients. Despite significantly greater myocardial injury in the control group, there were no differences in indices of cardiac output and systemic perfusion such as mixed venous saturation and urine output. Because intensive care management is aimed at maintaining adequate cardiac output guided by these indices, the lack of a difference is not surprising. Consequently, the higher inotrope requirement at 3 and 6 h can be interpreted as reflecting a greater degree of myocardial dysfunction in the control group.

Study limitations. In this preliminary study of the effects of RIPC in a clinical setting, we purposely chose children undergoing surgical repair of a diverse range of congenital heart defects with a wide age range. Despite randomization of these patients, the mean age and weight of the control patients were greater; however, this did not reach statistical significance. A larger multicenter study of these effects will allow the possibly variable effects of RIPC in discrete subgroups of patients to be studied. This is important, as there is a wide inter-individual variation in, for example,

inflammatory cytokine responses, as illustrated by the current data. Because patients with both increased and decreased pulmonary blood flow were included in this study, this may have confounded our measurements of lung mechanics. Furthermore, although there are reports of the preconditioning properties of inhalational anesthetics and many other diverse stimuli, patients in this study were randomized to RIPC or control groups and all underwent the same anesthetic, CPB, and intensive care management protocols to minimize bias.

It is impossible to examine the optimal timing and administration of the ischemic preconditioning stimulus from the current study. We chose to replicate the stimulus shown to be effective in experimental models and our protocol shown to modify human neutrophil gene responses. The risk-benefit ratio of this therapy is so striking that further studies should be directed toward optimizing the stimulus, as well as the possible additional benefits from the second window of protection using RIPC.

Summary. We have demonstrated the myocardial protective effects of remote ischemic preconditioning using a simple noninvasive technique of four 5-min cycles of lower limb ischemia and reperfusion. These novel data support the need for a larger study of RIPC in patients undergoing cardiac surgery and the potential additional benefit afforded by second-window preconditioning.

Reprint requests and correspondence: Dr. Andrew Redington, Head, Division of Cardiology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada. E-mail: andrew.redington@sickkids.ca.

REFERENCES

1. Laskey WK, Beach D. Frequency and clinical significance of ischemic preconditioning during percutaneous coronary intervention. J Am Coll Cardiol 2003;42:998–1003.
2. Teoh LK, Grant R, Hulf JA, Pugsley WB, Yellon DM. The effect of preconditioning (ischemic and pharmacological) on myocardial necrosis following coronary artery bypass graft surgery. Cardiovasc Res 2002;53:175–80.
3. Leesar MA, Stoddard MF, Xuan YT, Tang XL, Bolli R. Nonelectrocardiographic evidence that both ischemic preconditioning and adenosine preconditioning exist in humans. J Am Coll Cardiol 2003;42: 437–45.
4. Lindhardt TB, Gadsboll N, Kelbaek H, et al. Pharmacological modulation of the ATP sensitive potassium channels during repeated coronary occlusions: no effect on myocardial ischemia or function. Heart 2004;90:425–30.
5. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993;87:893–9.
6. Pell TJ, Baxter GF, Yellon DM, Drew GM. Renal ischemia preconditions myocardium: role of adenosine receptors and ATP-sensitive potassium channels. Am J Physiol 1998;275:H1542–7.
7. Gho BC, Schoemaker RG, van den Doel MA, Duncker DJ, Verdouw PD. Myocardial protection by brief ischemia in noncardiac tissue. Circulation 1996;94:2193–200.
8. Kharbanda RK, Mortensen UM, White PA, et al. Transient limb ischemia induces remote ischemic preconditioning in vivo. Circulation 2002;106:2881–3.
9. Konstantinov IE, Li J, Cheung MMH, Shimizu M, Kharbanda RK, Redington AN. Remote ischemic preconditioning of the recipient reduces myocardial ischemia-reperfusion injury of the donor heart via a Katp channel-dependent mechanism. Transplantation 2005;79:1691–5.
10. Konstantinov IE, Arab S, Kharbanda RK, et al. The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. Physiol Genomics 2004;19:143–50.
11. Kharbanda RK, Li J, Konstantinov IE, et al. Remote ischemic preconditioning protects from cardiopulmonary bypass injury in vivo. Circulation 2003;108:IV509.
12. Kharbanda RK, Li J, Konstantinov IE, et al. Remote ischemic preconditioning protects against cardiopulmonary bypass induced tissue injury—a preclinical study. Heart 2006. In press.
13. Novak I, Davies PS, Elliott MJ. Noninvasive estimation of total body water in critically ill children after cardiac operations. Validation of a bioelectric impedance method. J Thorac Cardiovasc Surg 1992;104: 585–9.
14. Wernovsky G, Wypij D, Jonas RA, et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. Circulation 1995;92:2226–35.
15. Shore S, Nelson DP, Pearl JM, et al. Usefulness of corticosteroid therapy in decreasing epinephrine requirements in critically ill infants with congenital heart disease. Am J Cardiol 2001;88:591–4.
16. Modi P, Imura H, Angelini GD, et al. Pathology-related troponin I release and clinical outcome after pediatric open heart surgery. J Card Surg 2003;18:295–300.
17. Cain BS, Meldrum DR, Dinarello CA, et al. Tumor necrosis factor-alpha and interleukin-1-beta synergistically depress human myocardial function. Crit Care Med 1999;27:1309–18.
18. Worrall NK, Chang K, LeJeune WS, et al. TNF-alpha causes reversible in vivo systemic vascular barrier dysfunction via NO-dependent and -independent mechanisms. Am J Physiol 1997;273:H2565–74.
19. Natanson C, Eichenholz PW, Danner RL, et al. Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med 1989;169:823–32.
20. Walley KR, Hebert PC, Wakai Y, Wilcox PG, Road JD, Cooper DJ. Decrease in left ventricular contractility after tumor necrosis factor-alpha infusion in dogs. J Appl Physiol 1994;76:1060–7.
21. Donnelly RP, Freeman SL, Hayes MP. Inhibition of IL-10 expression by IFN-gamma up-regulates transcription of TNF-alpha in human monocytes. J Immunol 1995;155:1420–7.
22. Barnas GM, Watson RJ, Green MD, et al. Lung and chest wall mechanical properties before and after cardiac surgery with cardiopulmonary bypass. J Appl Physiol 1994;76:166–75.
23. Schulze-Neick I, Penny DJ, Rigby ML, et al. L-arginine and substance P reverse the pulmonary endothelial dysfunction caused by congenital heart surgery. Circulation 1999;100:749–55.
24. Schulze-Neick I, Li J, Penny DJ, Redington AN. Pulmonary vascular resistance after cardiopulmonary bypass in infants: effect on postoperative recovery. J Thorac Cardiovasc Surg 2001;121:1033–9.
25. Pearl JM, Nelson DP, Wellmann SA, et al. Acute hypoxia and reoxygenation impairs exhaled nitric oxide release and pulmonary mechanics. J Thorac Cardiovasc Surg 2000;119:931–8.
26. Kharbanda RK, Peters M, Walton B, et al. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. Circulation 2001; 103:1624–30.
27. Li G, Chen S, Lu E, Luo W. Cardiac ischemic preconditioning improves lung preservation in valve replacement operations. Ann Thorac Surg 2001;71:631–5.
28. Chaturvedi RR, Lincoln C, Gothard JW, et al. Left ventricular dysfunction after open repair of simple congenital heart defects in infants and children: quantitation with the use of a conductance catheter immediately after bypass. J Thorac Cardiovasc Surg 1998;115: 77–83.